

L3 ANSWER 21 OF 23 CAPLUS COPYRIGHT 1999 ACS
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TI Production of insulin-like growth
factor-1 (IGF-1) in methylotrophic yeast cells
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PA Salk Institute Biotechnology/Industrial Associates, Inc., USA
SO PCT Int. Appl., 100 pp.
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DT Patent
LA English

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	CA 2090969	AA	19920305	CA 91-2090969	19910904
	EP 548267	A1	19930630	EP 91-918262	19910904
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	JP 06500470	T2	19940120	JP 91-516963	19910904
	US 5324639	A	19940628	US 93-23463	19930225
	US 5612198	A	19970318	US 94-308196	19940919
PRAI	US 90-578728		19900904		
	WO 91-US6452		19910904		
	US 93-983523		19930303		

TI Production of insulin-like growth
factor-1 (IGF-1) in methylotrophic yeast cells
AB Expression vectors and host cells contg. same are provided that stably
express IGF-1 and that secrete high concns. of biol. active
IGF-1; the expression system is readily scaled up to produce large
quantities of IGF-1. IGF-1 is produced by growing
methylotrophic yeast transformants contg. in their genome .gtoreq.1 copy
of DNA encoding IGF-1 in operational linkage with a signal
sequence which includes the proteolytic processing site Lys-Arg and may
include .gtoreq.1 Glu-Ala sequences. In preferred embodiments, the
signal

sequence is of the *Saccharomyces cerevisiae* .alpha.-mating factor pre-pro
sequence. Expression of both the DNA encoding IGF-1 and the
pre-pro signal sequence are regulated by a promoter region derived from a
methanol-responsive gene of a methylotrophic yeast. DNA constructs and
recombinant methylotrophic yeast strains used for the expression and
secretion of IGF-1 are also provided. For preferred
embodiments, protease-deficient *Pichia pastoris* strains are
provided. Fermen. and characterization of the IGF-1 secreted
into the culture medium (including amino acid compn. and sequence) are
described. High-level prodn. of IGF-1 by *P. pastoris*
was readily scaled up to a 10-L fermen. level.

ST insulin like growth factor 1
yeast; somatomedin C prodn methylotrophic yeast; *Pichia* somatomedin C
recombinant prodn

IT Plasmid and Episome

(DNA for insulin-like growth
factor 1 on, for insulin-like
growth factor 1 prodn. in methylotrophic yeast)

IT Deoxyribonucleic acid sequences

(for insulin-like growth factor
1 DNA of human)

IT Protein sequences
(for **insulin-like growth factor**
1 of human)

IT Deoxyribonucleic acids
RL: BIOL (Biological study)
(for **insulin-like growth factor**
1 prodn. in methylotrophic yeast)

IT **Pichia pastoris**
(**insulin-like growth factor** 1
recombinant prodn. in)

IT Fermentation
(of **insulin-like growth factor**
1, in **Pichia pastoris**)

IT Plasmid and Episome
(pIGF204, DNA for **insulin-like growth**
factor 1 on, for **insulin-like**
growth factor 1 prodn. in methylotrophic yeast)

IT Plasmid and Episome
(pIGF206, DNA for **insulin-like growth**
factor 1 on, for **insulin-like**
growth factor 1 prodn. in methylotrophic yeast)

IT Plasmid and Episome
(pIGF816, DNA for **insulin-like growth**
factor 1 on, for **insulin-like**
growth factor 1 prodn. in methylotrophic yeast)

IT **Saccharomyces cerevisiae**
(.alpha.-mating factor pre-pro sequence from, for signal sequence for
insulin-like growth factor 1
prodn. in methylotrophic yeast)

IT Gene, microbial
RL: BIOL (Biological study)
(AOX1, methanol-responsive gene of methylotrophic yeast and
transcription terminator derived from, of **Pichia pastoris**,
for **insulin-like growth factor**
1 recombinant prodn.)

IT Yeast
(methylotrophic, **insulin-like growth**
factor 1 recombinant prodn. in)

IT Plasmid and Episome
(pIGF201, DNA for **insulin-like growth**
factor 1 on, for **insulin-like**
growth factor 1 prodn. in methylotrophic yeast)

IT Plasmid and Episome
(pIGF202, DNA for **insulin-like growth**
factor 1 on, for **insulin-like**
growth factor 1 prodn. in methylotrophic yeast)

IT Genetic element
RL: BIOL (Biological study)
(promoter, from methanol-responsive gene of methylotrophic yeast, for
insulin-like growth factor 1
recombinant prodn.)

IT Microbial hormones and pheromones
RL: BIOL (Biological study)
(.alpha.-factor, of **Saccharomyces cerevisiae**, pre-pro sequence from,
for signal sequence for **insulin-like growth**
factor 1 prodn. in methylotrophic yeast)

IT 67-56-1, Methanol, uses
RL: USES (Uses)
(-responsive gene, promoter from, of methylotrophic yeast, for
insulin-like growth factor 1
recombinant prodn.)

IT 29586-66-1 141098-54-6 141098-55-7 141170-32-3
RL: PRP (Properties)
(DNA encoding processing sequence of, in **insulin-like**
growth factor 1 recombinant prodn. in methylotrophic

yeast)
IT 66795-41-3, **Insulin-like growth factor I** (human reduced) 97199-09-2
RL: BIOL (Biological study)
(amino acid sequence of and recombinant expression of DNA for,
complete)
IT 144132-72-9, Deoxyribonucleic acid (human clone pIGF101 **insulin-like growth factor I**-specifying)
RL: BIOL (Biological study)
(nucleotide sequence and expression in *Pichia pastoris* of,
complete)
IT 144132-71-8, Deoxyribonucleic acid (human clone pIGF101 **insulin-like growth factor I**-specifying plus 5'- and
3'-flanking region fragment)
RL: BIOL (Biological study)
(nucleotide sequence of and expression in *Pichia pastoris* of)
IT 67763-96-6P, **Insulin-like growth factor 1**
RL: PREP (Preparation)
(prodn. of, in methylotrophic yeast)
IT 9001-92-7, Protease 9046-67-7, Carboxypeptidase Y 37228-80-1
37288-81-6
RL: BIOL (Biological study)
(*Pichia pastoris* deficient for, in **insulin-like growth factor 1** recombinant prodn.)

L3 ANSWER 22 OF 23 TOXLIT
AN 1993:32458 TOXLIT
DN CA-118-095582H
TI Genes influencing proteolytic activity in *Pichia*, and protein manufacture
with protease-deficient *Pichia*.
AU Gleeson MA; Howard BD
SO (1992). PCT Int. Appl. PATENT NO. 92 17595 10/15/92 (Salk Institute
Biotechnology).
CY United States
DT Patent
FS CA
LA English
OS CA 118:95582
EM 199305
AB Genes involved with proteolysis in *Pichia* are cloned, and *Pichia* strains
with these genes inactivated are provided for manuf. of
proteinase-sensitive proteins. The genes for proteinase A (PEP4) and
proteinase B (PRB-1) and the orotidine-5'-phosphate decarboxylase gene
(URA3) of *P. pastoris* were cloned and sequenced. PEP4-URA3- *P.*
pastoris Strains were prep'd. and a gene for **insulin-like growth factor 1** (IGF1) introduced. These
strains produced more IGF1 than did strains contg. the wild-type PEP4
gene.

L3 ANSWER 23 OF 23 TOXLIT
AN 1993:32002 TOXLIT
DN CA-118-095126N
TI purification of intact, correctly-folded **insulin-like growth factor-1**.
AU Holtz GC; Brierley RA
SO (1992). PCT Int. Appl. PATENT NO. 92 12993 08/06/92 (Salk Institute
Biotechnology/Industrial Associates, Inc.).
CY United States
DT Patent
FS CA
LA English
OS CA 118:95126
EM 199305
TI purification of intact, correctly-folded **insulin-like**

growth factor-1.

AB Intact, correctly-folded, monomeric **insulin-like growth factor-1** (**IGF-1**) is recovered and purified by 2 series of chromatog. on cation exchange exchange and hydrophobic interaction adsorbents ending with gel filtration chromatog. Human **IGF-1** was purified from the fermn. broth of recombinant **Pichia pastoris** by cation exchange chromatog. on Sp-250 ZetaPrep or Toyopearl SP550C, hydrophobic interaction chromatog. on TosoHaas Bu Toyopearl-650M, cation exchange chromatog. on Fast Flow S-Sepharose, a chromatog. on the 650M resin, and gel filtration chromatog. on Superdex 75 or Toyopearl HW50F.

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